

THE AUTOLYSIS OF NITROGENOUS COMPOUNDS OF THE BLOOD SERUM IN GENERAL PAR- ALYSIS AND DEMENTIA PRAECOX

WITH ITS BEARING ON THE ABDERHALDEN TEST *

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In previous communications¹ from this laboratory it was demonstrated that the blood serum from normal animals, treated according to the Abderhalden method, with substrates prepared from various tissues, gave positive reactions, thus proving that disease of the corresponding organs is not necessary for the occurrence of a positive Abderhalden reaction with such substrates. It was further shown that both with animal and human serums the appearance of a positive reaction varied with the thoroughness with which the substrates were washed with boiling water. All substrates used could, by repeated washing, be rendered incapable of producing positive Abderhalden tests. It was further established that the serum of persons suffering from general paralysis of the insane, when tested with brain substrate, showed a quantitatively different reaction from that of normal individuals. This difference, calculated by personal estimates of the degree of ninhydrin reaction, was illustrated by a curve which is here reproduced in Figure 1.

In order to establish this fact by quantitative methods we have performed a series of tests with brain substrates and the blood serums of normal persons, of patients presenting unmistakable evidence of general paralysis of the insane and others showing a dementia praecox type of reaction.

Estimations of the amount of amino-acid nitrogen were made, by the Van Slyke method, in the serum immediately after separation and again at the end of twenty-four hours, during which the samples were kept in a shaking machine at a temperature of 37 C. These samples included serum alone and serum mixed with 0.25 gm. of brain substrate. At the end of the period the serum was separated from the substrate by filtration. In Table 1 are given the figures thus obtained and the average results are shown in graphic form in Figure 2 for convenience of comparison with the results shown in Figure 1.

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* From the Laboratory of the Illinois State Psychopathic Institute.

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1. Ross and Singer: *THE ARCHIVES INT. MED.*, 1914, xiv, 552; *Ibid.*, 1915, xv, 724.

These results seem to suggest very strongly that the coagulable nitrogen of the substrate can have nothing to do with the determination of a positive Abderhalden test, a conclusion which is strongly supported by the fact, previously published, that the materials removed from various organs by washing with slightly acidulated boiling water in the process of preparing the organs for substrates will themselves serve as efficient substrates for the production of a positive Abderhalden reaction.

TABLE 1.—INCREASE OF AMINO-ACID NITROGEN IN MILLIGRAMS PER CUBIC CENTIMETER OF BLOOD SERUM WHEN INCUBATED FOR TWENTY-FOUR HOURS AT 37 C.

	Donor of Serum	Before Incubation	Increase After 24 Hours Without Substrate	Increase After 24 Hours with Brain Substrate Which Had Been Washed*			
				2 Times	4 Times	6 Times	11 Times
Normal.....	1	0.886	0.023	0.096	0.057	0.012	0.021
	2	0.604	0.009	0.111	0.038	0.020
	Average	0.745	0.016	0.103	0.057	0.025	0.021
General paralysis of the insane	E. B.	0.520	0.001	0.168	0.028	0.028
	A. C.	0.796	0.008	0.073	0.059	0.012
	A. S.	0.732	0.018	0.116	0.046	0.001
	G. M.	0.597	0.007	0.220	0.188	0.099
	J. L.	0.399	0.026	0.192	0.122	0.110	0.085
	Average.....	0.609	0.012	0.154	0.122	0.087	0.045
Dementia praecox...	S. B.	0.604	0.019	0.186	0.060	0.041
	C. D.	0.684	0.020	0.154	0.028	0.028
	J. S.	0.815	0.014	0.074	0.052	0.030	0.000
	A. B.	0.794	0.010	0.074	0.037	0.004	0.000
	F. W.	0.596	0.019	0.126	0.056	0.040	0.045
	Average.....	0.699	0.016	0.123	0.048	0.033	0.023

* The water which had been used for the second and subsequent washings gave no reaction with ninhydrin.

Investigations were next undertaken of the antitryptic titer of serum, during which it was noted that the addition of placenta substrate to serum resulted in a considerable increase in the total nitrogen of the serum. It was found that this increase reached its maximum in about ten minutes. The substrate used was placenta which had been washed twice. The wash water was negative to ninhydrin and a negative result was obtained in the dialysate when this substrate was incubated with water or normal salt solution according to the Abderhalden technic.

To a number of tubes containing 0.25 gm. of this placenta were added 1 c.c. of blood serum. Of these tubes, one was immediately filtered after dilution to 20 c.c., a second was filtered at the end of ten minutes and the others after thirty, and 180 minutes respectively, the same dilution being made in each case. Kjeldahl determinations

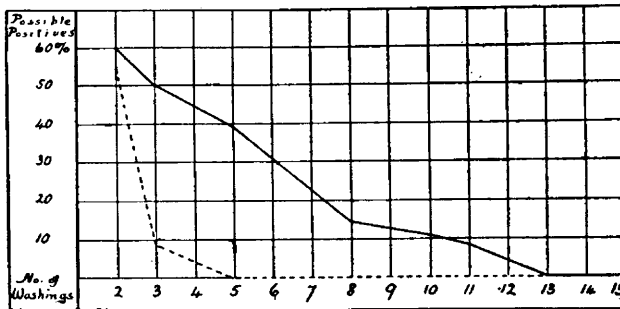


Fig. 1.—Proteolysis of brain substrate at different stages of washing by serum from normal persons and from patients with general paralysis. The solid line represents the results in general paralysis; the broken line, normal serum.

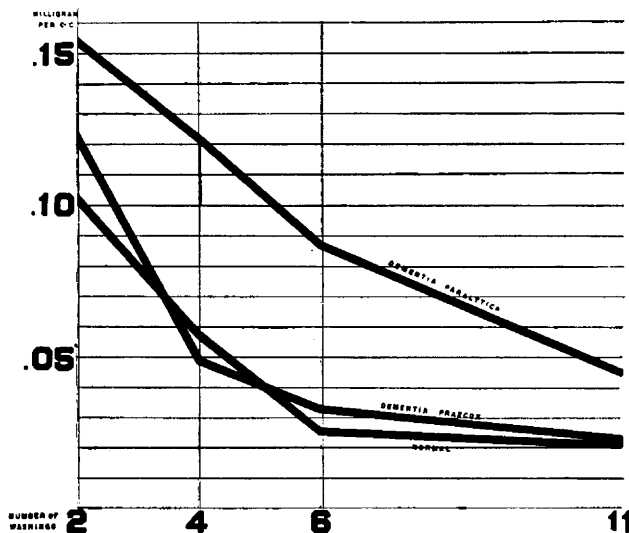


Fig. 2.—Increase of amino-acid nitrogen in serums incubated with brain substrate in various stages of washing.

were made on the blood serum alone, 0.25 gm. placenta alone, the filtrates from each of the above tubes and the residue with the filter paper in each instance. From these data it was possible to determine the increase in the total nitrogen of 1 c.c. of serum.

It was therefore decided to perform a series of tests on different blood serums and to compare the total autolysis of serum which had been treated for ten minutes with placenta substrate and then filtered with that which occurred in serum not so treated. The time allowed for the digestion was in all cases twenty-four hours and the method of determination used was that for noncoagulable nitrogen of Folin and Denis.² The total nitrogen of the serum was also determined by the Kjeldahl method. The results with normal, general paralytic and dementia praecox serums are given in Table 3.

TABLE 2.—INCREASE OF TOTAL NITROGEN OF SERUM FROM THE ADDITION OF PLACENTA SUBSTRATE

	Nitrogen, Mg.	Increase, Mg.
Original serum	11.653	
After momentary mixture with placenta.....	13.104	1.451
After 10 minute mixture with placenta.....	14.040	2.387
After 30 minute mixture with placenta.....	14.132	2.479
After 180 minute mixture with placenta.....	14.040	2.387

Figure 3 is a graphic representation of the average results for the three groups of blood serums given in Tables 1 and 3, those from the former being taken from the column for brain substrate which had been washed twice. The solid black represents the results from incubation of the serum alone, while the full height of the columns corresponds with those in serum which had been treated with substrate. It should be remembered in considering this chart that the first three columns refer to amino-acid nitrogen, the second three to total noncoagulable nitrogen and that the substrates used in the two sets were brain and placenta respectively.

In discussing these findings, one of the first points for especial emphasis is the fact that placental substrate allowed to act for only ten minutes is sufficient to remove antitryptic substances to such an extent that the increase in noncoagulable nitrogen during incubation for twenty-four hours exceeds that which occurs without the use of the substrate in serum from normal persons and from dementia praecox patients by approximately 260 per cent., and in general paralysis by 1,260 per cent. Furthermore, from the conditions of the experiment it is obvious that the nitrogen compounds which are split up must be those of the blood serum itself. The nitrogen derived from the substrate has, in each instance, been deducted from the figures here given for total noncoagulable nitrogen.

2. Jour. Biol Chem., 1911-1912, xi, 527.

The further observations, alluded to above, that the water which has been used for washing substrates contains materials capable of giving rise to positive Abderhalden tests and that substrates can be washed so thoroughly that they are no longer efficient, make it extremely probable that the active agent in removing antitryptic bodies from the serum is some soluble substance or substances. Necessarily,

TABLE 3.—INCREASE OF TOTAL NONCOAGULABLE NITROGEN IN MILLIGRAMS PER CUBIC CENTIMETER OF BLOOD SERUM WHEN INCUBATED FOR TWENTY-FOUR HOURS WITHOUT, AND AFTER, TREATMENT FOR TEN MINUTES WITH PLACENTA SUBSTRATE WHICH HAD BEEN WASHED TWICE, THE WASH WATER BEING NEGATIVE TO NINHYDRIN

	Donor of Serum	Total Nitrogen in Serum	Noncoagulable Nitrogen		
			Before Incubation	Increase after Twenty-Four Hours without Substrate	Increase after Twenty-Four Hours Subsequent to Treatment with Placenta for Ten Minutes Only
Normal.....	1	13.199	1.102	0.183	0.295
	2	14.793	0.938	0.069	0.332
	3	13.642	0.766	0.119	0.374
	4	13.465	0.903	0.154	0.333
Average.....	...	13.775	0.927	0.131	0.335
General paralysis of the insane	A. S.	11.288	1.346	0.000	0.197
	N. P.	12.667	1.047	0.071	0.392
	H. S.	13.376	1.065	0.003	0.448
	C. W.	13.908	0.964	0.011	0.265
	E. M.	14.137	1.033	0.043	0.340
Average.....	...	13.075	1.091	0.026	0.328
Dementia praecox.....	A. C.	15.375	1.152	0.082	0.076
	G. C.	14.174	0.975	0.244	0.634
	T. H.	14.085	0.898	0.064	0.292
	R. B.	14.571	0.970	0.174	0.325
Average.....	...	14.551	0.970	0.128	0.322

the concentration of these materials present in the wash water will vary according to the amount of water used in relation to the bulk of tissue being washed, the thoroughness of previous washings and the amount of these substances present in the tissue. The efficiency of the wash water will thus vary and we have found that it may be increased by careful evaporation. Some preliminary observations have been made with the wash water from placental tissue which seem sufficiently

significant to warrant mention here, although they are not by any means complete or exhaustive.

In one sample of such wash water it was found by the Kjeldahl method that nitrogen was present to the amount of 34.515 mg. per 100 c.c. Both urea and ammonium salts were present and determinations made of these two bodies gave 25.13 mg. urea nitrogen and 13.60 mg. ammonia nitrogen per 100 c.c., making a total of 38.73 mg. per 100 c.c., slightly larger than the Kjeldahl estimate for total nitrogen. It therefore seemed probable that the nitrogen present existed very largely in these two forms.

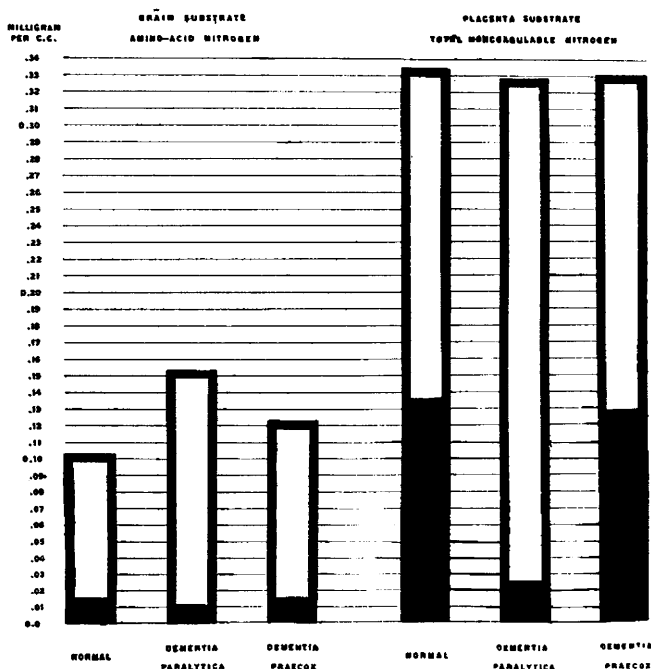


Fig. 3.—Effect of the removal of antitryptic substances from blood serums on the increase during incubation of amino-acid and of total noncoagulable nitrogen. Increases in the untreated serum are indicated in solid black, while those in the treated serum are represented by the full height of the columns.

Dialysis³ tests have been performed with blood serum mixed with placenta substrate, a solution of urea (1 c.c. contained 2.158 mg. of nitrogen), a solution of ammonium chlorid (1 c.c. contained 0.990 mg. of nitrogen) and a mixture of these last two. The tubes were

3. The dialyzing thimbles used were made of celloidin and we are not yet satisfied that they are free from objection.

incubated for sixteen hours and 10 c.c. of the dialysate tested with ninhydrin. The following results were obtained:

Serum alone	Negative
Serum with placenta	Positive
Serum with urea	Negative
Serum with ammonium chlorid	Positive
Serum with urea and ammonium chlorid.....	Positive

The positive reaction in each instance was faint and about equally so in all three. The serum used was that of a male general paralytic. It should be stated that in another test a positive was obtained with urea.

Attention may be called to certain other features in the tables and charts. The close similarity between dementia praecox and normal serum in all tests, with the wide divergence of that from general paralysis, is striking. Of course it is not possible to state without actual test that this is true for all substrates, but this seems at least probable if we are correct in regarding the substrate merely as a means of removing antitryptic properties.

It may be questioned, on the same grounds, whether we are justified in comparing the results of tests with placenta with those obtained when brain substrate is used. But it may be pointed out that the figures given offer a possible explanation for the fact that positive Abderhalden reactions are obtained with some serums and not with others. It will be observed that the ratio of the amino-acid nitrogen in the first set of tests to that of total noncoagulable nitrogen in the second set considered as 100 is as follows:

	Normal	General Paralysis	Dementia Praecox
Without substrate	13.0	46.0	12.5
With substrate	30.8	47.0	37.0

Amino-acid nitrogen gives a positive ninhydrin reaction; the lower products of lysis probably do not under the ordinary conditions of the Abderhalden test. Thus the blood serum of paretics not only has a higher antitryptic value, but, when this is neutralized or partly neutralized, the process of proteolysis takes place either less rapidly or less completely. The serum from dementia praecox also shows a somewhat greater proportion of amino-acid nitrogen and would therefore tend to develop a slightly larger percentage of positive Abderhalden reactions than the normal.

A comparison between the antitryptic titer of the different groups of serum, as measured by the two sets of tests, yields very strikingly similar results. The proportion of amino-acid nitrogen increase without substrate treatment to that with is 15.5 per cent. for the normal, 7.8 per cent. for general paralysis and 13 per cent. for dementia praecox. Corresponding figures for total noncoagulable nitrogen are 37.1

per cent., 20.3 per cent., and 38.5 per cent., respectively. If these are expressed in the form of ratios with general paralysis as 10, the results are as follows:

	Normal	General Paralysis	Dementia Praecox
Amino-acid nitrogen	20	10	17
Total noncoagulable nitrogen..	18	10	19

The number of cases examined is small and therefore broad conclusions are not advisable, but attention may be directed, in a further comparison of these three groups of serums, to the relation of non-coagulable nitrogen to total nitrogen. For both normals and dementia praecox it is 6.7 to 100 (although the figures are higher for dementia praecox), whereas in paresis this ratio is 8.4 to 100. Comparing the figures in Table 3 with those in Table 1, the percentage of noncoagulable nitrogen represented by amino-acid nitrogen, is as follows:

	Per Cent.
Normal	79.7
Dementia praecox	72
General paralysis	56

SUMMARY

1. The efficiency of brain substrate in destroying antitryptic bodies in blood serum, as estimated by determination of the amino-acid nitrogen produced, is gradually removed by repeated washing with boiling water.

2. Substrates prepared from placenta contain water-soluble substances capable of neutralizing antitryptic bodies. A sufficient amount of these substances can be extracted by the serum during ten minutes contact to permit free autolysis.

3. In the cases examined the average antitryptic titer of the blood serum was high for general paralysis and approximated the normal for dementia praecox.

4. After neutralization of antitryptic bodies the increase of non-coagulable nitrogen was approximately the same for normals, general paralytics and dementia praecox patients, but the proportion of amino-acid nitrogen was much greater in general paralysis and slightly greater in dementia praecox than in the normal.

5. The second wash water from placenta substrate contained urea and ammonium nitrogen, but was negative to ninhydrin. Positive Abderhalden tests have been obtained with ammonium chlorid as a substrate.

6. The noncoagulable nitrogen of the serum is increased by ten minutes' contact with placenta substrate.

CONCLUSIONS

Substrates neutralize antitryptic bodies in the blood serum, thus permitting fermentation of the nitrogenous constituents of the serum. This autolysis will, if not carried too far, cause a positive Abderhalden reaction.

The Abderhalden test is of no value as an index of disease or disorder of function in any particular organ.